RESEARCH ARTICLE

Solid Renal Tumor Severity in von Hippel Lindau Disease is Related to Germline Deletion Length and Location

Jodi K. Maranchie,[1] Anoushka Afonso,[1] Paul S. Albert,[4] Sivaram Kalyandrug,[6] John L. Phillips,[1] Shubo Zhou,[1] James Peterson,[1] Bijan M. Ghadimi,[2] Katheen Hurley,[1] Joseph Riss,[3] James R. Vasselli,[1] Thomas Ried,[2] Berton Zbar,[7] Peter Choyke,[5] McClellan M.Walther,[1] Richard D. Klausner,[3] and W. Marston Linehan[1*]

[1]Urologic Oncology Branch, National Cancer Institute, Bethesda, Maryland; [2]Genetics Branch, National Cancer Institute, Bethesda, Maryland; [3]Laboratory of Biosystems and Cancer, National Cancer Institute, Bethesda, Maryland; [4]Biometric Research Branch, National Cancer Institute, Bethesda, Maryland; [5]Radiology Department, National Institutes of Health, Bethesda, Maryland; [6]Capital Technology Information Services (CTIS), Inc., Bethesda, Maryland; [7]Laboratory of Immunobiology, National Cancer Institute, Frederick, Maryland

Communicated by Gregg L. Semenza

von Hippel Lindau disease (VHL) is an autosomal dominant familial cancer syndrome linked to alteration of the *VHL* tumor suppressor gene. Affected patients are predisposed to develop pheochromocytomas and cystic and solid tumors of the kidney, CNS, pancreas, retina, and epididymis. However, organ involvement varies considerably among families and has been shown to correlate with the underlying germline alteration. Clinically, we observed a paradoxically lower prevalence of renal cell carcinoma (RCC) in patients with complete germline deletion of *VHL*. To determine if a relationship existed between the type of *VHL* deletion and disease, we retrospectively evaluated 123 patients from 55 families with large germline *VHL* deletions, including 42 intragenic partial deletions and 13 complete *VHL* deletions, by history and radiographic imaging. Each individual and family was scored for cystic or solid involvement of CNS, pancreas, and kidney, and for pheochromocytoma. Germline deletions were mapped using a combination of fluorescent in situ hybridization (FISH) and quantitative Southern and Southern blot analysis. An age-adjusted comparison demonstrated a higher prevalence of RCC in patients with partial germline *VHL* deletions relative to complete deletions (48.9 vs. 22.6%, p=0.007). This striking phenotypic dichotomy was not seen for cystic renal lesions or for CNS (p=0.22), pancreas (p=0.72), or pheochromocytoma (p=0.34). Deletion mapping revealed that development of RCC had an even greater correlation with retention of HSPC300 (C3orf10), located within the 30-kb region of chromosome 3p, immediately telomeric to *VHL* (52.3 vs. 18.9%, p <0.001), suggesting the presence of a neighboring gene or genes critical to the development and maintenance of RCC. Careful correlation of genotypic data with objective phenotypic measures will provide further insight into the mechanisms of tumor formation. Hum Mutat 23:40–46, 2004. © **2003 Wiley-Liss, Inc.**

KEY WORDS: VHL; germline deletion; phenotype; genotype; renal neoplasms; HSPC300; C3orf10

DATABASES:
VHL – OMIM: 193300; GenBank: NM_000551.1 (mRNA), AC034193 (ganomic)
C3orf10 (HSPC300) – UniGene Cluster: Hs.421654; GenBank: AF161418.1 (mRNA)

INTRODUCTION

von Hippel Lindau (VHL) disease is an autosomal dominant cancer syndrome linked to inactivating alterations of the *VHL* tumor suppressor gene (MIM# 193300). Affected individuals are predisposed to develop highly vascular tumors of multiple organs, including clear cell tumors of the kidney, hemangioblastomas of the central nervous system (CNS), and pheochromocytomas. The clinical spectrum of these tumors varies widely from family to family, and evidence suggests that the specific *VHL* alteration influences the phenotype [Chen et al., 1995; Hes et al., 2000]. By defining these genotype–phenotype correlations, we hope to further map the discrete tumor suppressor functions of *VHL*. Our experience caring for families with von Hippel Lindau disease has led to the observation that patients with complete germline deletion of the *VHL* gene tend toward a less severe clinical

Received 13 March 2003; accepted revised manuscript 20 August 2003.

*Correspondence to: W. Marston Linehan, M.D., Urologic Oncology Branch, CCR, Bldg 10 Rm 2B47, National Cancer Institute, Bethesda, Maryland 20892-1501. E-mail: uob@mail.nih.gov

DOI 10.1002/humu.10302
Published online in Wiley InterScience (www.interscience.wiley.com).

presentation than patients with partial *VHL* deletions. To determine if there is indeed a relationship between the type of *VHL* deletion and severity of disease, we retrospectively examined tumor size and frequency in 123 patients from 55 families with partial or complete *VHL* deletion. We found that partial deletion patients did have a significantly higher frequency of renal cell carcinoma (RCC) than complete deletion patients; we did not see statistically significant differences for other organ systems. Further, chromosomal mapping of the deletions revealed that development of RCC best correlated with retention of the 30-kb region immediately telomeric to *VHL*, suggesting the presence of one or more genes involved in the development or maintenance of RCC.

MATERIALS AND METHODS

Clinical Evaluation

Patients with suspected or confirmed von Hippel Lindau syndrome were screened in our outpatient clinic. Evaluation included history and physical examination, serum electrolytes, complete blood count, liver function tests, and serum and urine catecholamines. Radiographic screening included contrast-enhanced CT or MRI of the abdomen and pelvis, and contrast-enhanced MRI of the brain and spine. Informed consent was obtained from each patient, and additional studies were performed when clinically indicated. Peripheral blood from one member of each lineage was analyzed for alterations or deletions of the *VHL* gene, as previously described [Stolle et al., 1998]. Between 1993 and 1999, 55 separate deletions lineages were identified; 42 harbored partial deletions, and 13 had complete germline deletions of the *VHL* gene.

Objective Phenotypic Index

Imaging studies obtained at initial visit to our institution were reviewed by one radiologist (PC). Tumors of the kidney, CNS, pancreas, and adrenal gland were counted and measured in two dimensions, and each organ was scored for both tumor frequency and total tumor volume for that one point in time. Information regarding prior surgery for each organ was extracted from intake history on chart review.

Molecular Mapping

Peripheral blood was obtained from one member of each lineage, and genomic DNA was extracted per protocol (Puregene; Gentra Systems, Minneapolis, MN). Quantitative Southern analysis was performed as previously described [Stolle et al., 1998]. Briefly, 5 μg DNA were cut with EcoRI and AseI, separated electrophoretically, denatured, transferred, and cross-linked to a nylon membrane (Hybond N, Amersham, www.amershambiosciences.com). The HSPC300 (GenBank# AF161418.1) probe was prepared by RT PCR using primers: 5′-GCATGGCGGGACAGGAGGATCC-3′ and 5′-GAGGAGACAAAGACTGTTCAC-3′, and radiolabeled using the Deca Primer II kit, as instructed (Ambion; www.ambion.com). Washed blots were exposed to a PhosphorImager Storm (Molecular Dynamics; www.amershambiosciences.com) screen overnight, stripped with 0.25% SDS, and reprobed using radiolabeled beta-globin cDNA. HSPC300 band intensity was normalized to beta-globin intensity, then divided by the normalized intensity seen when two HSPC300 alleles are present. Ratios of less than 0.58 were read as haploid at the HSPC300 locus. Northern analysis for HSPC300 was performed using total RNA extracted from cell lines as described [Gnarra et al., 1996] using the same HSPC300 probe and multiple tissue RNA blots (Clontech; www.clontech.com). For exon mapping, blots were prepared in an identical fashion, then probed with radiolabeled cDNA representing each of the three full-length exons, as well as the full length *VHL* mRNA. Exon boundaries derived from genomic sequence in BAC clone AC034193.

Chromosomal Mapping

Peripheral blood from one member of each kindred was processed for metaphase chromosomes as described [Veldman et al., 1997]. FISH was performed using cDNA probes prepared from chromosome 3p cosmid clones 3, 8, 11, and 31, phage P1-191, and λX36; and scored for monosomy at each locus, as previously described [Moch et al., 1998; Phillips et al., 2001].

Statistical Methods

The statistical analyses focused on comparing phenotypic measurements (i.e., prevalence, tumor frequency, and tumor volume) across groups defined by germline sequence variation. Because a number of patients had prior surgery to an organ system, prevalence was defined as the probability of either having a solid tumor at the initial scan or having had surgery to that organ system, expressed as a percentage of the entire population.

Estimates of prevalence as well as mean tumor volume and frequency were obtained both by averaging values across individuals and by averaging family-specific means. Average log-transformed tumor frequency and volume were obtained by adding 0.5 to all measurements, transforming to the log-scale, and averaging across individuals. Various statistical methods were used in accounting for familial correlation when testing for differences in the phenotypic distributions across groups. The type of statistical method depended on the distribution of the outcome variable (e.g., prevalence, frequency, or volume). Group differences in prevalence were tested using generalized estimating equations (with a binary outcome and a logit link function) with a Wald test [Liang and Zeger, 1986]. Group differences in tumor frequency were tested using a generalized linear mixed model (with Poisson outcome and a log link function) with a Wald test [Breslow and Clayton, 1993]. Group differences in total tumor volume, which for some organ systems contained many zeros, were tested by comparing the median family volume using Wilcoxon rank tests (two groups) and Kruskal-Wallace ANOVA tests (more than two groups). An adjustment for age (in quartiles) was made in all statistical analyses. For the prevalence and frequency analyses, age adjustment was made by including covariates in the models. For the volume analyses, age adjustment was done by forming an age-adjusted standardized residual and comparing the median family residual across groups using Wilcoxon rank and Kruskal-Wallace tests. The age distribution between groups was compared using a linear mixed model to account for the potential of family correlation. All tests are two-sided and $p < 0.05$ was considered statistically significant.

RESULTS

VHL is a classic tumor suppressor and follows the two-hit hypothesis of tumor development [Knudson, 1995], requiring loss or inactivation of both alleles for tumor formation. Patients with familial VHL disease are predisposed to form tumors because they carry one inactivating alteration in the germline. Thus, we were intrigued to find that patients with complete germline deletion of *VHL* tended to have the least severe clinical disease, as defined by necessity for surgical intervention (Table 1). Between 1990 and 1999, we followed 13 families with complete deletion. Of those 13 families, 11 families demonstrated the attenuated phenotype,

TABLE 1. **Population of Complete and Partial VHL Germline Deletion Families**

	Complete deletion	Partial deletion	Total	p value
Number of families	13	42	55	–
Number of individuals	31	92	123	–
Median individuals per family (range)	2 (1–5)	1.5 (1–12)	–	–
Mean age at presentation (years)	38.3	34.1	–	p = 0.15
Individuals with solid CNS tumors	23 (74%)	70 (76%)	93 (75.6%)	p = 0.22
Individuals with solid renal tumors	7 (22.6%)	45 (49%)	52 (42%)	p = 0.007
Individuals with solid pancreatic tumors	3 (10%)	10 (11%)	13 (10.5%)	p = 0.72

whereas two families had particularly severe disease, requiring multiple resections for relatively fast-growing tumors. To determine what differentiated these families genotypically, we performed fluorescent in situ hybridization (FISH) on 11 of these families, including the two severe families. We also included a known partial *VHL* deletion family observed to have the same attenuated phenotype characteristic of complete deletion [Yoshida et al., 2000]. Figure 1 shows the FISH results over a 200-kb region of chromosome 3p, demonstrating that most of these families have large regions of 3p deleted, in addition to *VHL*. Note that deletion boundaries are estimated within $\pm$20-kb due to the resolution limits of FISH. The severe phenotype families, 10 and 11, are among the smaller deletions in this group, suggesting the possibility that phenotypic severity correlates with deletion size. Further inspection reveals that the two severe families share retention of a common region, 30 kb immediately telomeric to *VHL*, as indicated by the box in Figure 1. This raised the alternative hypothesis that this region harbors sequences critical to the development and/or maintenance of *VHL*-related tumors. Thus codeletion would result clinically in a decreased prevalence of tumors. Examination of this region reveals only one known gene, originally cloned by the Chinese Genome project, designated HSPC300 (GenBank# AF161418.1; currently designated C3orf10 by HUGO). The protein is highly conserved in human, rodent, Drosophila, and *C. elegans*, but there is no suggestion as to its function. Northern analysis demonstrated expression of HSPC300 in all tissues tested (Fig. 2), including kidney and pancreas.

To further examine the relative contributions of deletion size and loss of the neighboring telomeric region, we expanded our study to include 42 families known to carry partial germline deletions of *VHL* (Table 1). Each deletion was mapped by Southern analysis for each of the individual exons of *VHL*. Figure 3A shows a representative series of blots for four families. Note that two different size alleles are seen for all four patients when probed with the full length *VHL* cDNA. The exon 2 probe does not recognize the smaller allele of Patient 1, indicating that exon 2 has been deleted. Patients 2, 3, and 4 retain exons 1 and 2, but exon 3 is deleted. Mapping of all 42 partial deletion families in shown in

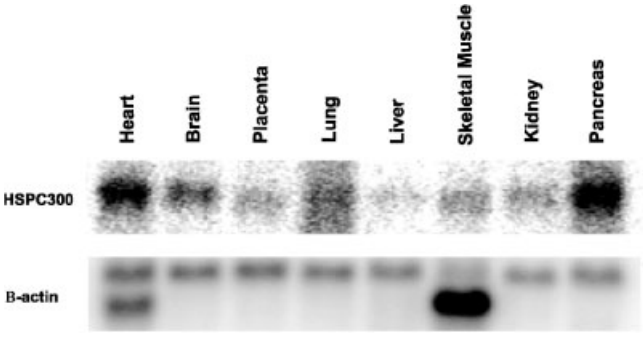

FIGURE 2. **Northern Blot analysis of Multiple Tissue Blot (Clontech) demonstrates expression of HSPC300 in all tissues examined, including kidney and pancreas.**

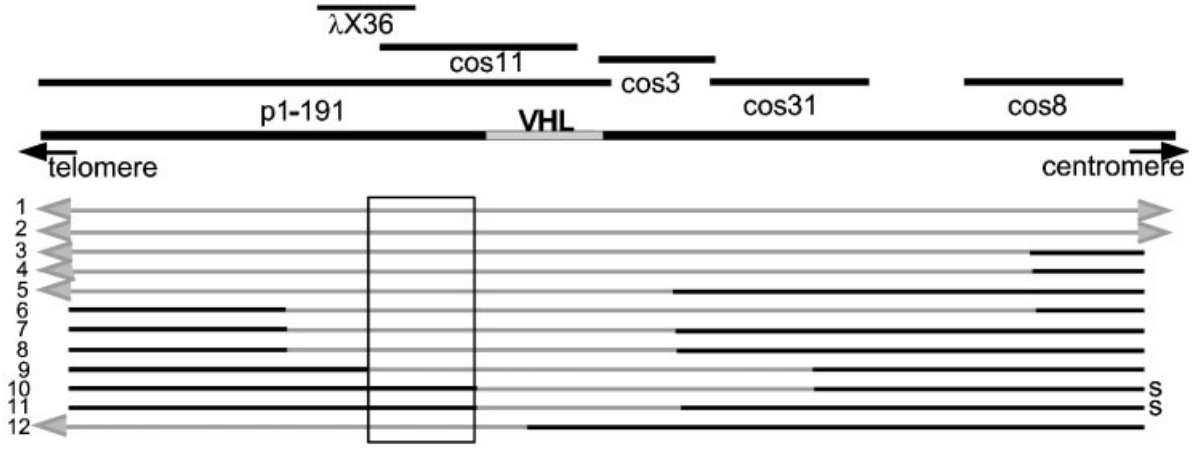

FIGURE 1. **FISH mapping on chromosome 3p for 11 complete deletion families and one partial deletion family (Family 12). Cut points are estimated within $\pm$ 20 kb. An S denotes patients with severe clinical disease. The location of overlapping phage and cosmid probes is indicated. Note that the two severe families share a common area of retention, 30 kb telomeric to VHL, as denoted by the box.**

Figure 3B, demonstrating a heterogeneous distribution, with representation of each exon deletion.

To identify deletions telomeric to *VHL*, we performed quantitative Southern analysis of each family, using the HSPC300 cDNA probe. Only 2 of 42 (5%) partial deletion families were found to have germline deletion of HSPC300 (Fig. 3B), including our attenuated Family 12 from Figure 1. Finally, to identify deletions extending centromeric to *VHL*, FISH was performed on nine of the 20 families with exon 3 deletion. No large centromeric deletions were discovered.

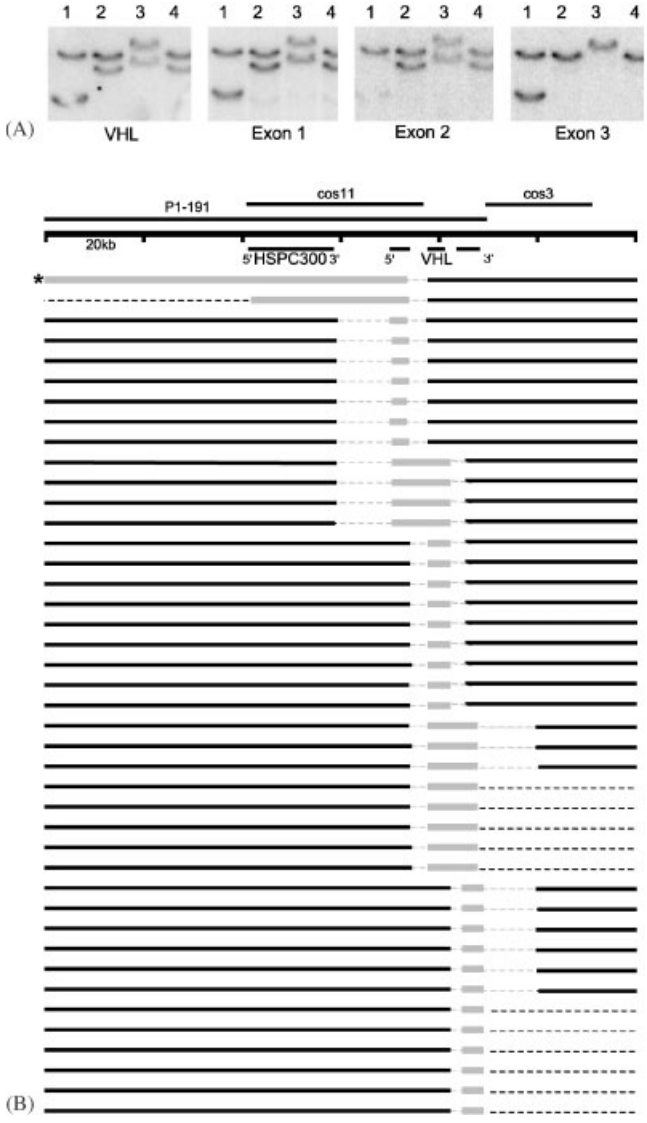

FIGURE 3. **Chromosomal mapping of partial VHL deletion families. A:** A series of genomic DNA Southern blots for four families. Note that two alleles of differing sizes are seen when probed with the full-length VHL cDNA. The smaller band for Family 1 is not recognized by an exon 2 cDNA probe indicating deletion of exon 2. Families 2–4 have deleted exon 3. **B:** Exon and HSPC300 (C3orf10) deletion mapping for all 42 partial deletion families. The asterisk indicates Family 12 from Figure 1. Grey bars indicate areas of deletion and black lines indicate retention. Gray dashed lines are regions of uncertainty. Areas indicated by black dashed lines were not evaluated.

Concurrent with the genotypic mapping, detailed phenotypic assessments were performed for all 123 individuals with complete or partial *VHL* deletion. First, each patient was scored as affected or unaffected by solid tumors of the CNS, pancreas, and kidney or by pheochromocytoma, based on imaging at first screening or history of surgical resection. Using this binary data set, comparison of complete and partial deletion patients (Fig. 4A) demonstrated a significantly increased prevalence of solid renal tumors in the partial deletion group (48.9 vs. 22.6%, $p=0.007$). Interestingly, this phenotypic dichotomy was specific to solid renal tumors, and not seen for pancreas (10.9 vs. 9.6%, $p=0.72$), CNS (76.1 vs. 74.2%, $p=0.22$), or pheochromocytoma (6.6 vs. 0%, $p=0.336$). When we grouped patients according to retention or deletion of HSPC300 (Fig. 4B), we found an even greater difference in the prevalence of RCC ($p \leq 0.001$). Four families crossed over in this analysis, two partial deletions and two complete deletions. It is important to note that the two partial deletion families with HSPC300 deletion were also the only two found to have deletions extending beyond *VHL*. In the absence of extended centromeric deletions in patients retaining HSPC300, we cannot rule out the possibility that the significance of HSPC300 deletion is due to deletion size. However, review of Figure 1 shows that Families 7 and 8 and carry deletions similar in size to Family 10, but codelete the region of HSPC300.

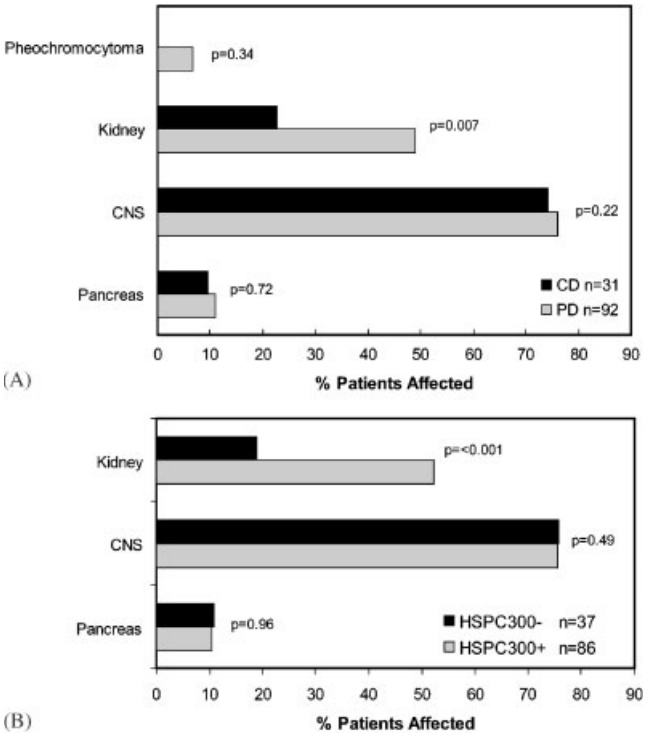

FIGURE 4. **Prevalence of organ involvement by solid tumors in 123 deletion patients. Organs were determined to be affected by radiographic imaging or history of surgical resection. A:** Comparison of partial and complete VHL deletion patients. **B:** Comparison of patients with or without retention of the neighboring telomeric gene, HSPC300.

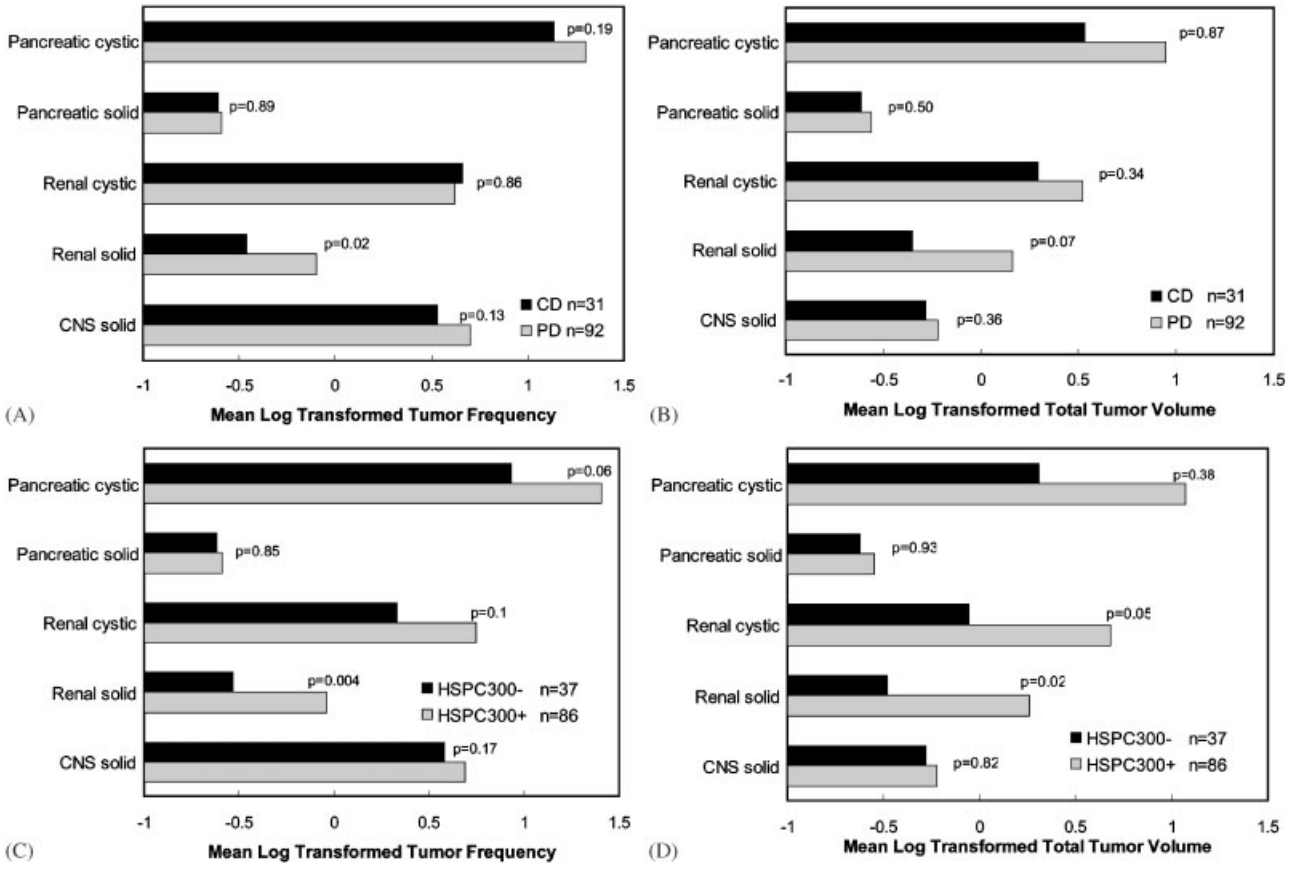

FIGURE 5. **Log-transformed tumor frequency and total volume in VHL deletion patients. A: Comparison of partial and complete VHL deletion patients by tumor frequency in each organ. B: Comparison of partial and complete VHL deletion patients by total tumor volume (in mm^3) in each organ. C: Comparison of HSPC300 retention vs. deletion by tumor frequency. D: Comparison of HSPC300 retention vs. deletion by total tumor volume (in mm^3) in each organ. The value for pancreatic cystic disease in the HSPC300+ population was –0.107 mm^3.**

To provide a more quantitative index of relative phenotypic severity, we next radiographically scored tumor frequency and total tumor volume for solid or cystic lesions in each organ. This new data set reflected one point in time—the imaging performed at first presentation—and did not account for prior surgical history. Using mean tumor frequency, we again saw a significantly increased prevalence of solid renal tumors in the partial deletion group (Fig. 5A). Although somewhat less significant ($p=0.02$), the results remained qualitatively similar, suggesting that tumor frequency at presentation provided a valid reflection of phenotypic severity. Interestingly, there was not a significant difference in total tumor volumes between patients with partial and complete deletions (Fig. 5B). This was possibly due to the fact that tumor size at presentation is highly variable and dependent more upon time of screening than actual tumor pathology. Despite this, the trend toward increased total volume of RCC frequency in partial deletion patients remained ($p=0.07$).

When the patients were regrouped according to HSPC300 status (Fig. 5C and D), the difference in RCC frequency increased ($p=0.004$), and there was a significant increase in total RCC volume with HSPC300 retention ($p=0.02$).

Finally, comparison of the different exon deletion groups yielded no significant differences (data not shown), likely due to the small number in each group. However, there was an increased frequency of RCC ($p=0.04$) in patients who retained exon 1 ($n=66$) vs. those with exon 1 deletion ($n=54$), possibly consistent with reports that alterations resulting in truncation of the VHL protein correlate with a higher prevalence of RCC [Yoshida et al., 2000].

DISCUSSION

VHL disease predisposes affected individuals to tumor formation in multiple organs, but the relative prevalence of manifestations varies widely from family to family and can be related to the underlying germline alteration. Up to 98% of patients affected by pheochromocytoma carry germline missense alterations [Chen et al., 1995], whereas nonmissense, or truncating alterations, correlate with development of both familial and sporadic RCC [Gnarra et al., 1994; Yoshida et al., 2000]. pVHL has recently been identified as the substrate recognition element of an E3 ligase targeting HIFalpha subunits for ubiquitin-mediated degradation. However, analysis of genotype–phenotype relationships suggests that the VHL

protein may have multiple, tissue-specific functions [Richards et al., 1998]. Although multiple genetic changes are required for tumor formation, consistent genotype–phenotype correlations provide the best human model for the functional sequelae of specific *VHL* alterations and will enhance our understanding of the role of *VHL* in both familial and sporadic disease.

We demonstrate that the prevalence of RCC is significantly lower in patients with complete germline deletion of *VHL* than in patients with partial deletions. Further, this difference becomes even greater when patients are grouped according to retention or deletion of a representative telomeric gene, HSPC300. This striking phenotypic dichotomy is not seen for the other manifestations of VHL disease studied, including renal cysts, pheochromocytoma, CNS hemangioblastoma, or solid or cystic lesions of the pancreas, suggesting that the protective effect is specific to the role of *VHL* in RCC tumorigenesis.

Attenuated phenotype in complete deletion patients has not been previously reported. Hes et al. [2000] examined the phenotype of five *VHL* deletion families, including one complete deletion. Consistent with our observations, they found that both partial and complete deletion families have a very low prevalence of pheochromocytoma and a high prevalence of CNS disease. However, the small number of families did not permit identification of differences in the prevalence of pheochromocytoma between partial and complete deletions.

Several reports have demonstrated a correlation between nonmissense alterations, predicted to result in truncated or grossly altered pVHL, and the development of RCC [Friedrich, 2001]. One large analysis of 77 Japanese families found this correlation to be specific to RCC. Interestingly, they report a 74% prevalence of RCC for all nonmissense patients, which is considerably higher than the 50% we saw in partial deletion patients alone. They speculate that gross disruption or complete loss of pVHL may be critical to the development of RCC [Yoshida et al., 2000]. This is consistent with the finding that the majority of VHL alterations found in sporadic clear cell RCCs are nonmissense [Gnarra et al., 1994]. There are no reports, however, to suggest that complete deletion of *VHL* would actually abrogate development of RCC.

All of the patients with attenuated phenotype shared a common codeletion of the 30-kb region of chromosome 3p, immediately telomeric to *VHL*. This suggested that the region might harbor oncogenes involved in the genesis or maintenance of RCC. The only known gene in this region, HSPC300, codes for a 75–amino acid, highly conserved protein with no known function. If expression of HSPC300 does support tumor growth, then loss of one or both alleles would be expected to abrogate development of RCC. Preliminary studies overexpressing HSPC300 in VHL-deficient tumor cells demonstrated no effect on growth in culture, and coimmunoprecipitation revealed no direct interaction between HSPC300 and VHL or interference with VHL binding of elongin B, elongin C, or Cul-2 (data not shown).

Alternatively, HSPC300 may not support RCC, but may serve a fundamental housekeeping gene, critical to cell survival. The incidence of loss of heterozygosity (LOH) involving large regions of chromosome 3p is greater than 95% in RCC [Gnarra et al., 1994], whereas LOH is documented in only 62% of CNS hemangioblastomas [Glasker et al., 2001] and is rarely seen in renal cysts [Phillips et al., 2001]. Increased LOH in RCC is consistent with reports of frequent LOH at 3p21 [Alimov et al., 2000], 3p12, and 3p14 [Lubinski et al., 1994], regions believed to contain secondary tumor suppressors lost in stepwise renal tumor progression [Lott et al., 2002]. Thus, if LOH is requisite for renal malignant transformation, but also results in loss of the second allele of a critical housekeeping gene, then this second hit would result in cell death rather than renal tumor growth.

Alternatively, as mentioned above, our results do not rule out the possibility that the RCC protective effect is due to overall deletion size rather than location. The attenuated phenotype deletions, which involved deletion of HSPC300, were also the largest deletions in this study. It is possible that subsequent LOH results in excessive loss of genetic material and cell death, not specifically due to the region of HSPC300. Further, our data do not rule out the theoretic possibility that larger deletions may predispose the loss of fetuses having interacting genetic variants at other loci, thereby decreasing the prevalence of solid renal tumors within the kindred.

In summary, we report a significantly decreased prevalence of RCC in VHL patients who carry germline deletions of the 30-kb region immediately telomeric to *VHL*. The majority (85%) of complete *VHL* deletions codelete this region. This striking phenotypic dichotomy is specific to solid renal tumors and is not seen for the other manifestations of VHL disease. Further investigation of this protective phenomenon will continue to provide insight into the mechanism of renal tumorigenesis and may yield important targets for tumor prevention.

REFERENCES

Alimov A, Kost-Alimova M, Liu J, Li C, Bergerheim U, Imreh S, Klein G, Zabarovsky ER. 2000. Combined LOH/CGH analysis proves the existence of interstitial 3p deletions in renal cell carcinoma. Oncogene 19:1392–1399.

Breslow NE, Clayton DG. 1993. Approximate inference in generalized linear models. J Am Stat Assoc 88:9–25.

Chen F, Kishida T, Yao M, Hustad T, Glavac D, Dean M, Gnarra JR, Orcutt ML, Duh FM, Glenn G, Green J, Hsia YE, Lamiell J, Li h, Wei MH, Schmidt L, Tory K, Kuzmin I, Stackhouse T, Latif F, Linehan WM, Lerman M, Zbar B. 1995. Germline mutations in the von Hippel-Lindau disease tumor suppressor gene: correlations with phenotype. Hum Mutat 5:66–75.

Friedrich CA. 2001. Genotype-phenotype correlation in von Hippel-Lindau syndrome. Hum Mol Genet 10:763–767.

Glasker S, Bender BU, Apel TW, van Velthoven V, Mulligan LM, Zentner J, Neumann HP. 2001. Reconsideration of biallelic inactivation of the VHL tumour suppressor gene in hemangioblastomas of the central nervous system. J Neurol Neurosurg Psychiatry 70:644–648.

Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, Latif F, Liu S, Chen F, Duh FM, Lubensky IA, Duan R, Florence C, Pozzatti R, Walther MM, Bander NH, Grossman HB, Brauch H, Pomer S, Brooks JD, Isacs WB, Lerman MI, Zbar B, Linehan WM. 1994. Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat Genet 7:85–90.

Gnarra JR, Zhou S, Merrill MJ, Wagner JR, Krumm A, Papavassiliou E, Oldfield EH, Klausner RD, Linehan WM. 1996. Post-transcriptional regulation of vascular endothelial growth factor mRNA by the product of the VHL tumor suppressor gene. Proc Natl Acad Sci USA 93:10589–10594.

Hes F, Zewald R, Peeters T, Sijmons R, Links T, Verheij J, Matthijs G, Leguis E, Mortier G, van der Torren K, Rosman M, Lips C, Pearson P, van der Luijt. 2000. Genotype-phenotype correlations in families with deletions in the von Hippel-Lindau (VHL) gene. Hum Genet 106:425–431.

Knudson AG. 1995. VHL gene mutation and clear-cell renal carcinomas. Cancer J Sci Am 1:180.

Liang KY, Zeger SL. 1986. Longitudinal data analysis using generalized linear models. Biometrika 73:12–22.

Loh KC, Shlossberg AH, Abbott EC, Salisbury SR, Tan MH. 1997. Phaeochromocytoma: a ten-year survey. QJM 90:51–60.

Lott ST, Chandler DS, Curley SA, Foster CJ, El-Naggar A, Frazier M, Strong LC, Lovell M, Killary AM. 2002. High frequency loss of heterozygosity in von Hippel-Lindau (VHL)- associated and sporadic pancreatic islet cell tumors: evidence for a stepwise mechanism for malignant conversion in VHL tumorigenesis. Cancer Res 62:1952–1955.

Lubinski J, Hadaczek P, Podolski J, Toloczko A, Sikorski A, McCue P, Druck T, Huebner K. 1994. Common regions of deletion in chromosome regions 3p12 and 3p14.2 in primary clear cell renal carcinomas. Cancer Res 54:3710–3713.

Moch H, Schraml P, Bubendorf L, Richter J, Gasser TC, Mihatsch MJ, Sauter G. 1998. Intratumoral heterogeneity of von Hippel-Lindau gene deletions in renal cell carcinoma detected by fluorescence in situ hybridization. Cancer Res 58:2304–2309.

Phillips JL, Ghadimi BM, Wangsa D, Padilla-Nash H, Worrell R, Hewitt S, Walther M, Linehan WM, Klausner RD, Ried T. 2001. Molecular cytogenetic characterization of early and late renal cell carcinomas in von Hippel-Lindau disease. Genes Chromosomes Cancer 31:1–9.

Richards FM, Webster AR, McMahon R, Woodward ER, Rose S, Maher ER. 1998. Molecular genetic analysis of von Hippel-Lindau disease. J Intern Med 243:527–533.

Stolle C, Glenn G, Zbar B, Humphrey JS, Choyke P, Walther M, Pack S, Hurley K, Andrey C, Klausner R, Linehan WM. 1998. Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. Hum Mutat 12:417–423.

Veldman T, Vignon C, Schrock E, Rowley JD, Ried T. 1997. Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. Nat Genet 15:406–410.

Yoshida M, Ashida S, Kondo K, Kobayashi K, Kanno H, Shinohara N, Shitara N, Kishida T, Kawakami S, Baba M, Yamamoto I, Hosaka M, Shuin T, Yao M. 2000. Germ-line mutation analysis in patients with von Hippel-Lindau disease in Japan: an extended study of 77 families. Jpn J Cancer Res 91:204–212.